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Increased Pulsatile, But Not Basal, Growth Hormone Secretion Rates and Plasma Insulin-Like Growth Factor I Levels during the Periovulatory Interval in Normal Women

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ABSTRACT

The secretion of GH changes during the menstrual cycle, exhibiting high levels during the periovulatory phase (PO). Previous studies have not investigated whether this difference in GH status is due to increased secretion or reduced clearance of pituitary GH and amplified pulsatile *vs.* basal GH secretion. It is also unclear whether the PO phase is accompanied by changes in circulating insulin-like growth factor I (IGF-I). In this study we investigated the 24-h GH release patterns in the early follicular (EF) *vs.* the periovulatory menstrual phase in the same individuals. Ten young (aged 24–34 yr) healthy women with regular menses were studied with deconvolution analysis of GH profiles obtained by blood sampling every 20 min for 24 h, followed by an arginine stimulation test. A high sensitivity immunofluorometric GH assay was used. All women were studied in both the EF and PO phases in random order. There were no differences in the basal GH secretion rate or GH half-life during the two phases. The number of GH secretory bursts identified during the 24-h sampling period was significantly increased during the PO (13.3 ± 0.5) compared to the EF (10.3 ± 0.6) phase ($P = 0.002$); conversely, the mean interburst interval was shorter in the PO (107 ± 5 min) than in the EF (134 ± 8 min) phase ($P = 0.004$). There was no difference in GH pulse mass ($P = 0.13$) or amplitude ($P = 0.21$) between the two phases. The pulsatile GH production rate (milligrams per L/24 h) was significantly elevated during the PO (61 ± 6) compared to that during the EF (37 ± 8; $P = 0.004$). Increased total GH pulse area was confirmed by Cluster analysis ($P = 0.027$). Furthermore, the 24-h mean serum GH concentration was significantly increased in the PO (1.4 ± 0.1 mg/L) *vs.* that in the EF (0.9 ± 0.1 mg/L; $P = 0.002$). There was a positive correlation between estradiol (E_2) and GH secretory pulse amplitude, frequency, and mean 24-h serum GH concentration in the PO cycle phase, indicating E_2 to be a major statistical determinant of GH secretion. Serum GH increased significantly after arginine infusion in both phases ($P < 0.001$), whereas there was no difference between the two cycle phases ($P = 0.20$). Serum IGF-I levels were increased during the PO phase (253 ± 20 mg/L) compared to those during the EF phase (210 ± 16 mg/L; $P = 0.03$), whereas serum IGF-binding protein-3, IGF-II, and GH-binding protein were similar during the two phases. This study unequivocally documents elevated GH levels during the PO phase of the menstrual cycle, mediated by increased GH production rate and burst frequency. The concomitant increase in serum IGF-I suggests a central stimulation of the GH-IGF-I axis, which may be mediated by endogenous E_2 levels. (*J Clin Endocrinol Metab* 83: 1662–1667, 1998)

THE INFLUENCE of gender and sex steroids on GH status has been extensively investigated, but controversies and unresolved issues remain (1). It is generally accepted that premenopausal women exhibit higher GH levels than comparably aged men regarding both spontaneous 24-h profiles and the response to many (but not all) GH stimulation tests. There is evidence to suggest that this gender-specific difference is secondary to differences in estradiol (E_2) levels. In a study of both young and older adults of both sexes, Ho *et al.* (2) reported serum E_2 to be the primary determinant of integrated 24-h GH concentrations rather than gender *per se* or age. By contrast, a recent study involving two age groups of both sexes demonstrated that abdominal adiposity, which was increased in males, was the most important and negative determinant of stimulated GH release (3). A specific effect of E_2 on GH status is, however, also inferred from studies demonstrating higher spontaneous and stimulated GH levels during the periovulatory (PO) phase of the menstrual cycle in normal young women (4). It has also been convincingly demonstrated that estrogen administration amplifies endogenous GH levels in girls with gonadal dysgenesis (5) and in postmenopausal women. The elevation in GH levels after exogenous estrogen is observed typically after oral, but not transdermal, administration, and there is evidence that only transdermal estrogen increases serum insulin-like growth factor I (IGF-I) levels, whereas oral administration results in reduced serum IGF-I levels, which is explained by a first pass inhibitory effect of estrogen on hepatic IGF-I production (6). It has, however, recently been shown that both routes of administration can increase GH levels and reduce the serum IGF-I concentration (7). Whether the amplifying effect of estrogen on serum GH levels is a primary effect or is secondary to an inhibition of IGF-I pro-

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duction, therefore, remains unresolved. It is also unknown whether the estrogen-associated increased GH levels are solely due to increased pituitary GH secretion, or whether changes in the kinetics of GH removal also occur, as has been suggested to account for the higher serum GH concentrations in women compared to those in men (8). It also remains controversial whether sex steroids influence the patterned regularity of 24-h GH profiles in the human.

To investigate the mechanisms subserving the putative E_2-associated increase in GH concentrations in the late follicular phase, we applied the following strategies: 1) a homogeneous group of young healthy premenopausal women was studied, each individual being assessed during two phases of her menstrual cycle to minimize interindividual variation; 2) deconvolution analysis and approximate entropy were used to allow estimation of production rates and patterned regularity of GH in addition to mean GH levels and peak numbers; 3) an arginine stimulation test was performed on each occasion to allow a comparison between spontaneous and stimulated GH levels; and 4) the levels of IGF-I and IGF-binding protein-3 (IGFBP-3) were measured to evaluate the effects of sex steroids on the entire GH-IGF-I axis.

Subjects and Methods

Subjects (Table 1)

Ten healthy young women with regular menstrual cycles occurring every 27–29 days were examined. All subjects were nonobese according to body mass index, waist/hip ratio (W/H), and percent lean body mass (Table 1). All women were studied twice in random order: in the early follicular (EF) phase, days 2–5 after the onset of menstrual bleeding; and in the PO phase, days 13–15 after the onset of menses. Two subjects had distinctly elevated P values during the PO phase, suggesting early postovulation. These two subjects exhibited differences in GH levels that were qualitatively similar to the others. The tests were separated by at least one normal menstrual cycle. Volunteers underwent 24-h blood sampling followed by an arginine stimulation test. All subjects gave their written informed consent, and the study was approved by the local ethics committee and conducted according to the Declaration of Helsinki.

24-h GH profile

After an overnight fast, the subjects were admitted to the hospital at 0830 h, and an iv cannula was placed in a antecubital vein. Blood sampling was started at 0900 h and continued every 20 min for 24 h. Serum was separated and stored at −20 C until assayed. Subjects were given three meals per day and were allowed normal physical activity but refrained from sleeping during the day. No record of sleep was obtained.

Arginine stimulation test

The test was performed after completion of each 24-h GH sampling profile, and the subjects remained supine during the test. At 0900 h, arginine (0.5 g/kg BW) was infused iv over 30 min, and blood samples were withdrawn every 10 min for 2 h. One of the 10 women only completed 1 arginine stimulation test, so in the paired statistics only 9 women are included.

TABLE 1. Characteristics of the subjects (n = 10)

Age (yr)	28.4 ± 1
Ht (m)	1.65 ± 2
Wt (kg)	61.5 ± 3
BMI (kg/m^2)	22.4 ± 1
LBM (%)	73.6 ± 1
Waist/Hip (W/H) ratio	0.78 ± 0

Hormone analysis

A double monoclonal immunofluorometric assay (Delfia, Wallac, Turku, Finland) was used to measure GH. The lower detection limit was 0.01 mg/L. Serum IGF-I and IGF-II were determined after acid-ethanol extraction by a novel in-house immunofluorometric method using two monoclonal antibodies (9). Dose-dependent within-sample variance was calculated in each 24-h time series using all 73 replicated (duplicated) samples and was used in the pulse analysis. Serum concentrations of FSH, LH, PRL, and progesterone were measured using commercially available RIA kits. Serum concentrations of E_2 were analyzed by RIA using the Spectria kits from Orion Diagnostica (Espoo, Finland). The intra- and interassay coefficients of variation were 7.3% and 5.1%, respectively. Serum concentrations of GH-binding protein (GHBP) were measured using a ligand immunofunctional assay (10).

Body composition

Percent body fat and total lean body mass were assessed using bioelectrical impedance (Animeter, HTS, Odense, Denmark) (11). Body mass index was defined as the subject's weight in kilograms divided by the square of her height in meters. The W/H ratio was defined as the ratio between the circumference of the waist at the umbilicus and that of the hips at throchanter major.

Deconvolution analysis

A pulsatile model of hormone secretion and clearance was assumed, in which the plasma concentration of GH at any given instant is related to four simultaneous secretory and kinetic features of interest: 1) the locations, 2) the amplitudes, and 3) the durations of significant GH secretory bursts, acted upon continuously by 4) an endogenous subject-specific hormone half-life, as previously described (12). A basal (time-invariant) rate of GH secretion was calculated simultaneously to reflect the lowest 5% of sample GH concentrations in any given profile. A distinct secretory burst was defined algebraically as an approximately random (Gaussian) distribution of instantaneous molecular secretory rates, in which the fitted amplitude could be distinguished from zero with 95% certainty. A convolution integral was used to relate the serum GH concentrations to the foregoing specific measure of pulsatile GH secretion and removal, which were quantified by iterative nonlinear least squares parameter estimation. The disappearance function for GH was modeled as a one-component exponential decay function with a subject-specific rate constant (12), assuming that the half-life and distribution volume of GH were approximately constant in each individual throughout the 24 h.

The above deconvolution analysis estimates 1) the daily (endogenous) pulsatile production rate (milligrams per L GH distribution volume/24 h); product of mass per burst and number of bursts (total secretion includes any basal secretion, here found to be approximately zero); 2) secretory burst amplitude (milligrams per L/min); maximal rate of secretion attained within the computed secretory event (mass per distribution volume/min), 3) mass secreted per burst (milligrams per L); area of the calculated secretory burst; hence, amount of hormone secreted per burst per U distribution volume; 4) number of bursts; and 5) $t_{1/2}$ of (endogenous) GH disappearance (minutes).

All analyses were carried out blinded to the order of admissions.

Cluster analysis

The Cluster analysis (13) was included as a model independent analysis.

Approximate entropy (ApEn) statistic

Normalized ApEn is a scale- and model-independent statistic for assessing the regularity of time-series data. It assigns a single nonnegative number to a time series, quantifying an idea of the serial orderliness or regularity of the data. ApEn measures the logarithmic likelihood that runs of patterns of data length m that are similar remain similar within

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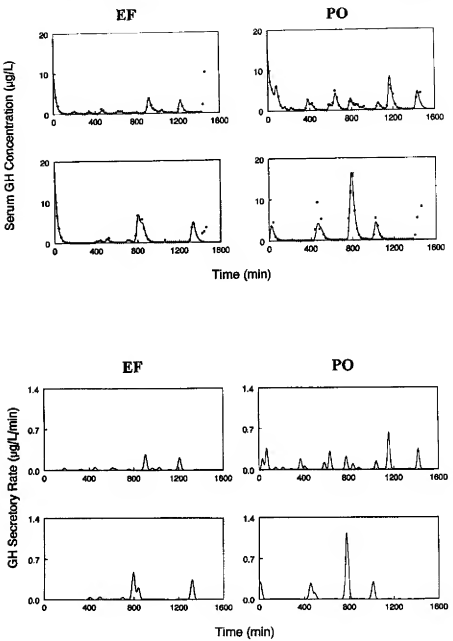

FIG. 1. *Top panel*, Illustrative profiles of fitted GH concentrations from two subjects during the EF phase (*left panel*) and matching PO phase (*right panel*). *Lower panel*, Illustrative profiles of GH secretion curves from the same two subjects during the EF phase (*left panel*) and matching PO phase (*right panel*).

a tolerance r on next incremental comparisons. Smaller ApEn values indicate a greater likelihood of successive comparisons remaining close and therefore imply greater regularity, and *vice versa*. It has been demonstrated that ApEn is very stable to repeated small changes in noise characteristics or infrequent, albeit large, data artifacts. The calculation of ApEn has been thoroughly described (14). Two input parameters, m and r, must be fixed to compute ApEn; m is the length of compared runs, and r is effectively a tolerance or filter. In this study, $m = 1$, and $r = 20\%$ of the SD of each GH time series, which serves to normalize ApEn to any differences in absolute serum GH concentrations. These ApEn parameters have been shown to define statistically and biologically significant contrasts in the orderliness of hormone release in various 24-h time series (15–17).

Statistical analysis

Comparisons among the variables between the two menstrual phases were made using paired Student's t test and Wilcoxon nonparametric test. Multiple linear regression was used to detect correlations between E_2, FSH, LH, IGF-I, CHBP, age, W/H, and fat-free mass (independent variables) and estimates of GH secretion and half-life derived from deconvolution analysis (dependent variables). ANOVA was used to test for changes within and between the two phases during the arginine stimulation test. In addition, the area under the curve (AUC) and the difference between baseline and maximum level postarginine administration were compared by paired t tests. Data are given as the mean ± SEM. Statistical significance was assumed for $P < 0.05$.

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Results

24-h GH profile (Figs. 1 and 2)

The basal secretion of GH was similar during the EF and PO phases (EF, 0.001 ± 0.0 mg/L·min; PO, 0.002 ± 0.0; P = 0.19). Expressed as daily rates, these values are 1.4 and 2.8 mg/L, respectively, which are less than 5% of the pulsatile daily secretion rates and, hence, were omitted from further analysis. The mean 24-h serum GH concentration was significantly higher in the PO than in the EF phase (EF, 0.87 ± 0.1 mg/L; PO, 1.35 ± 0.1; P = 0.004). Furthermore, the mean estimated production rate of GH was increased during the PO phase (EF, 37.0 ± 7.7 mg/L·24 h; PO, 60.6 ± 6.0; P = 0.01). The calculated GH secretory burst amplitude was similar during the two phases (EF, 0.12 ± 0.02 mg/L·min; PO, 0.15 ± 0.02; P = 0.13), whereas the number of GH secretory bursts was significantly increased during the PO phase compared to that during the EF phase [EF, 10.3 ± 0.6 (number of bursts per 24 h); PO, 13.3 ± 0.5; P = 0.004], and thus, the GH interburst interval was significantly shorter in the PO phase than in the EF phase (EF, 134.4 ± 8 min; PO, 107.4 ± 5; P = 0.006). No difference was seen between the two phases in either the mass of GH secreted per burst (EF, 3.7 ± 0.8 mg/L; PO, 4.7 ± 0.6; P = 0.19) or the GH half-life (EF, 17.6 ± 0.8 min; PO, 17.0 ± 0.4; P = 0.92). Cluster analysis revealed a higher maximal peak height (P = 0.037) and total pulse area (P = 0.027) during the PO phase, whereas the peak number did not significantly differ between the two phases. Furthermore, there was no difference between ApEn values during the EF phase compared to those during the PO phase (EF, 0.51 ± 0.04; PO, 0.54 ± 0.05; P = 0.56). Similar ApEn results were obtained when the calculation was repeated after first differencing to remove 24-h trends.

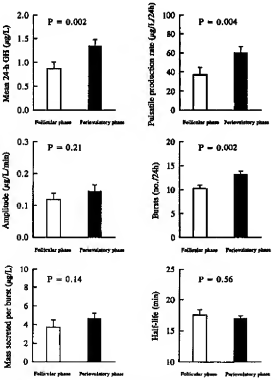

FIG. 2. Quantitative characteristics of endogenous GH secretion and half-lives during the EF and PO phase derived from deconvolution analysis. The data are the mean ± SEM.

Arginine stimulation test (Fig. 3)

Serum GH increased significantly in both phases after arginine infusion (P < 0.001), whereas there was no difference between the two cycle phases (P = 0.20). This was also reflected in the AUCs, expressed as mean GH levels during arginine stimulation (EF, 7.28 ± 1.5 μg/L; PO, 9.51 ± 1.9; P = 0.20). Furthermore, the differences between baseline and maximum level postarginine administration were similar on the two occasions (EF, 12.6 ± 2 mg/L; 15.4 ± 3; P = 0.32).

Other serum hormone levels and pertinent associated variables (Table 2)

Serum E_2 and LH were significantly higher in the PO phase than in the EF phase, whereas serum progesterone and FSH were comparable during the two phases. Serum GHBP levels was similar during the two phases. Serum IGF-I, IGF-II, and IGFBP-3 were measured at 0900, 1500, and 2100 h, and the results reported are the means of these three measurements. Serum IGF-I was significantly increased during the PO phase compared to that during the EF phase. The calculated product of the mean GH concentration and the IGF-I concentration (EF, 191.93 ± 46; PO, 342.32 ± 44; P = 0.002) further increased the difference between the EF and PO phases. Serum IGFBP-3 and IGF-II levels were comparable.

Correlations between estimates of GH secretion and different hormone levels, age, and body composition

By multiple regression analysis with features of GH status as the dependent variable and sex steroids, gonadotropins, GHBP, IGF-I, and age as independent variables, no significant determinants of GH status were disclosed in the EF

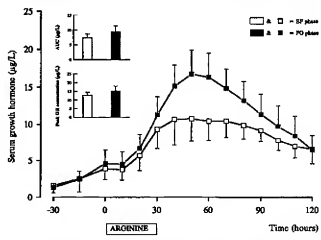

FIG. 3. Serum GH during arginine GH stimulation test in the EF phase (□) and the PO phase (■). *Inset*, AUCs, expressed as mean GH levels during arginine stimulation (*upper panel*) and peak GH concentrations (*lower panel*).

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TABLE 2. Serum hormone levels and pertinent associated variables in the EF and the PO menstrual phases

Parameter	EF	PO	*P* value
FSH (IU/L)	23.1 ± 2	18.8 ± 1	0.10
LH (IU/L)	5.0 ± 1	10.3 ± 2	0.03
Estradiol (nmol/L)	0.15 ± 0	0.50 ± 0	0.001
Progesterone (nmol/L)	1.79 ± 1	11.1 ± 5	0.12
IGF-I (μg/L)	210 ± 16	253 ± 20	0.03
IGFBP-3 (μg/L)	3171 ± 210	3139 ± 176	0.89
IGF-II (μg/L)	1229 ± 53	1175 ± 56	0.42

phase. By contrast, in the PO phase the serum E_2 concentration was able to significantly predict GH secretory status in most equations. With GH production rate as the dependent variable, the positive association with E_2 was significant, with a level of significance between 0.045–0.009 depending on the composition of the other independent variables. In some equations in the PO phase LH appeared as a positive, but weaker, determinant ($P = 0.08–0.02$) of GH secretory status. Finally, there was no correlation in either the EF phase or the PO phase with measures of body composition.

Discussion

In this study we investigated spontaneous and arginine-stimulated GH release in young women with regular menstrual cycles during the EF and PO phase. All participants were studied on both occasions in a paired (randomly ordered) design, and we used a deconvolution technique to unravel quantitative and qualitative features of GH secretion and half-life. Analysis of the 24-h profiles disclosed that the integrated serum GH concentration was significantly higher in the PO compared to the EF phase, which was attributed to an elevated GH production rate and increased number of secretory bursts. During the PO phase, the women exhibited approximately 30% more GH pulses than during the EF phase. There was no difference in the low rate of basal GH secretion, which accounted for less than 5% of the total daily GH secretion, or in the half-life of GH, nor was burst mass or amplitude different during the two phases. Furthermore, as a measure of the orderliness of the GH release process, approximate entropy values were alike in the two situations. We found a positive correlation between estimates of GH secretory status and both LH and E_2 during the PO phase. Regarding the arginine stimulation test, we found no significant differences in GH secretion between the two phases. Serum IGF-I levels were increased during the PO phase, whereas serum IGFBP-3, IGF-II, and GHBP levels were similar during the two phases.

Earlier studies of GH release in premenopausal women have focused on differences between young and older women and between sexes (18), whereas 24-h GH release during the menstrual cycle has only been investigated in a few studies. Our study partially confirms the study by Faria *et al.* (4) in which PO levels of mean 24-h GH were increased compared to those during the EF phase of the menstrual cycle, albeit not necessarily in the same women. In their study, which was carried out with a less sensitive GH assay and discrete peak detection without deconvolution analysis, this rise in GH was due to an increased GH pulse amplitude rather than number of pulses. In our study, we found no difference between GH secretory burst mass or amplitude, but there was a significantly increased number of GH secretory pulses. We observed 10.3 and 13.3 pulses/24 h in the EF and PO phases, whereas Faria *et al.* only detected 8.3 and 7.9 pulses/24 h in the same two phases. This difference probably reflects a greater sensitivity of GH pulse detection by way of both analytical technique (deconvolution *vs.* Cluster analysis) and assay threshold, especially given that earlier GH assays failed to detect low amplitude secretory bursts. Furthermore, Faria *et al.* studied different women during the different phases, whereas we investigated the same women during the two phases, thus eliminating interindividual differences. In addition to the higher frequency of GH pulses during the PO phase, the total pulsatile GH production rate was also found to be greater than that in the EF phase. This was not evaluated in the study by Faria *et al.*, but was suggested by increased total pulse area (as confirmed here by deconvolution and independently by Cluster analysis; data not shown). The difference in sampling rates between the two studies (20 and 10 min) could have some implications, but it has recently been shown that a 20-min sampling interval can allow significant information (16), including detection of estrogen-stimulated GH secretory burst frequency (5). As in the Faria study, we detected a positive correlation between E_2 and GH secretory pulse amplitude, production rate, and mean 24-h GH in the PO cycle phase and for the first time between E_2 and GH secretory burst frequency, collectively suggesting E_2 to be a major determinant of GH secretion. This is in accord with the results of studies in postmenopausal women treated with exogenous estrogen, in whom GH levels were increased after both oral and adequately dosed transdermal E_2 administration (7).

In an earlier study by Zadik *et al.* (19), 23 normal women were subjected to continuous 24-h sampling in the follicular and again in the luteal phase of the menstrual cycle, with no evident differences in the integrated concentrations of GH during the two phases. Comparable observations were reported in studies based on daily blood samples throughout the menstrual cycle (20–22), whereas others found a periovulatory rise in serum GH (23, 24). Ovulation induction was also found to result in a severalfold increase in serum GH concentrations during the PO period (25). These 6 studies are all based on single GH values in blood, which do not necessarily reflect endogenous GH secretion.

The observed lack of difference in arginine-stimulated GH release during the menstrual cycle is in accordance with the results of studies using exogenous GHRH as a GH secretagogue (26, 27), whereas higher GH levels in the PO phase have been reported after stimulation with arginine (28) and exercise (22). The reason for this ambiguity is unknown, but it could be a type 2 error in the negative studies.

The serum GHBP level did not differ between the two phases, which is in line with the findings of a previous study (29). It has recently been found that females secrete GH with more process irregularity than males (16) and in a similar immunofluorometric assay secrete more GH per pulse than men (18). Although we found no cycle-dependent difference in GH secretion pattern regularity as quantified by ApEn, estrogen treatment of girls with gonadal dysgenesis in-

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creased the disorderliness of GH release (higher ApEn) (30). As estrogen treatment in this context also increases GH pulse amplitude (5), our findings in the PO phase of higher GH pulse frequency and unchanged ApEn are readily distinguished from the GH axis response to exogenous estrogen.

Serum IGF-I levels were significantly increased in the PO phase by our paired comparisons, which contrasts with other recent studies that found no change in serum IGF-I levels throughout the menstrual cycle (29, 31, 32). In postmenopausal women receiving oral estrogen, amplification of endogenous GH levels is usually encountered together with reduced serum IGF-I levels (6). In that context it is, therefore, likely that the increased GH release represents a feedback response to the reduction in peripheral IGF-I levels comparable to what is observed during fasting (1). In contrast, and much like the combined increase in serum GH and IGF-I that occurs in pubertal *vs.* prepubertal girls (33), here during the PO phase we find evidence for GH hypersecretion relative to IGF-I levels, thus strongly suggesting central actions of estrogen to stimulate GH production via one or more mechanisms (33). This was also reflected in the calculated product of the mean GH concentration and the IGF-I concentration, which showed an increased value, whereas accommodation of high GH to low IGF-I levels would keep the product nearly constant. The mechanisms underlying this apparent divergence in IGF-I responses after exogenous *vs.* endogenous elevation in E_2 levels are unclear, but it has been suggested that oral estrogen directly inhibits hepatic IGF-I production as a first pass (pharmacological) effect (6).

In conclusion, this study shows that the elevated GH levels during the PO phase of the menstrual cycle are due to an increase in the pulsatile GH production rate and a rise in the GH secretory burst frequency. Furthermore, the concomitant increase in plasma IGF-I concentrations suggests that the ability of endogenous E_2 to amplify pituitary GH release is not secondary to the suppression of hepatic IGF-I production.

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